

REVIEW

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Tissue engineering strategies for spiral ganglion neuron protection and regeneration

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Abstract

Cochlear implants can directly activate the auditory system's primary sensory neurons, the spiral ganglion neurons (SGNs), via circumvention of defective cochlear hair cells. This bypass restores auditory input to the brainstem. SGN loss etiologies are complex, with limited mammalian regeneration. Protecting and revitalizing SGN is critical. Tissue engineering offers a novel therapeutic strategy, utilizing seed cells, biomolecules, and scaffold materials to create a cellular environment and regulate molecular cues. This review encapsulates the spectrum of both human and animal research, collating the factors contributing to SGN loss, the latest advancements in the utilization of exogenous stem cells for auditory nerve repair and preservation, the taxonomy and mechanism of action of standard biomolecules, and the architectural components of scaffold materials tailored for the inner ear. Furthermore, we delineate the potential and benefits of the biohybrid neural interface, an incipient technology in the realm of implantable devices. Nonetheless, tissue engineering requires refined cell selection and differentiation protocols for consistent SGN quality. In addition, strategies to improve stem cell survival, scaffold biocompatibility, and molecular cue timing are essential for biohybrid neural interface integration.

Introduction

Hearing loss impairs cognitive development, mainstream social integration, and maintenance of quality of life. In addition, the risk of dementia and Alzheimer's disease in elderly populations with hearing loss also increases [1]. Sensorineural hearing loss (SNHL) is usually caused by damage to the auditory sensory epithelium or auditory cortex afferent nerve pathways, including the degeneration or loss of hair cells (HCs) and spiral ganglion neurons (SGNs) [2]. The inherent repair ability of mature sensorineural tissue in the mammalian cochlea after injury is quite limited and insufficient to support spontaneous and effective regeneration. Therefore, the degeneration or loss of SGNs is permanent. The factors causing the loss of SGNs mainly include noise exposure, side effects of ototoxic drugs, infection, genetic factors, and aging (Graphical abstract image) [3–5].

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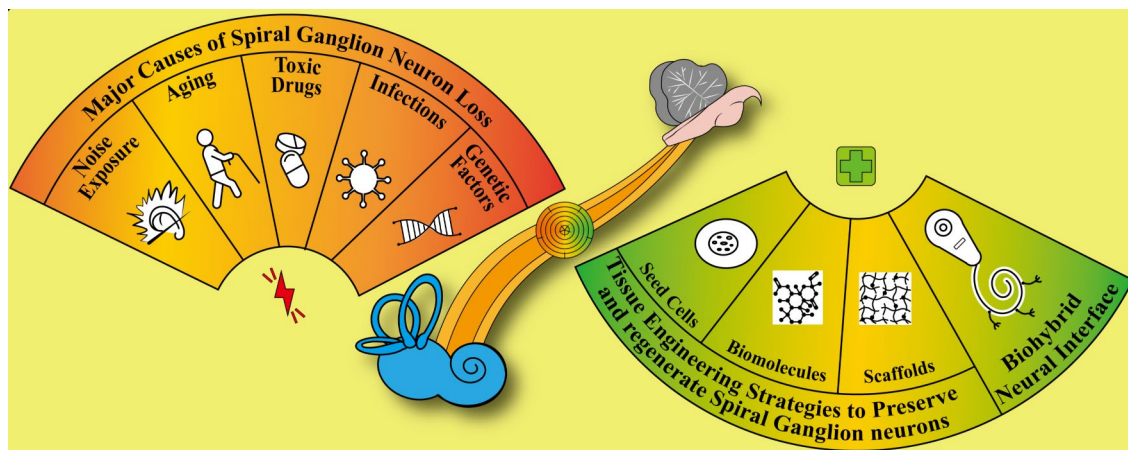
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Graphical Abstract

Keywords Spiral ganglion neurons, Tissue engineering, Sensorineural hearing loss, Biohybrid neural interface

SGNs located in Rosenthal's spiral canal can be divided into type I and type II afferent neurons (Fig. 1). As shown in Fig. 1B, most of them are type I, the cell body extends a peripheral process towards the organ of Corti, and a central process to the cochlear nucleus, which is responsible for transmitting auditory information from the inner hair cell (IHCs) to the auditory brainstem, and has different sensitivity to sound and spatial firing rate. The SGNs form synapses on the baso-lateral surface of hair cells at different positions along the basilar membrane, which underlies the topology of cochlear tones and enables the cochlea to encode sound intensities with a much wider dynamic range and facilitates speech recognition in noisy backgrounds [6]. A cochlear implant (CI) with direct stimulation of the auditory nerve is currently the only effective treatment for severe and profound SNHL; however, its efficacy is limited by the quantity and physiological condition of the remaining SGNs. Thus, the maintenance and regeneration of SGNs and their synapse are critical for SNHL therapy. The development of tissue engineering offers new hope for SNHL by integrating exogenous stem cells along with biomolecules on scaffolds, which on the one hand can provide nutritional support and guidance to the injured host nerve, and on the other hand can be integrated into the host as a neural substitute or relay [7, 8]. It is foreseeable that the new generation of biohybrid neural interfaces constructed by tissue engineering will greatly improve upon issues such as poor interface compatibility and narrow dynamic range of sound coding that exist in conventional CIs.

In this review, we synthesize the pivotal factors and underlying mechanisms contributing to SGN degeneration. Furthermore, we delineate the current state of SGN protection and the potential of stem cell replacement therapies. We comprehensively categorize and scrutinize

the role of biomolecules, along with an analysis of the utility and mechanisms of action of various bioactive molecules. Additionally, we survey the progression in the development of biomaterial scaffold systems designed to support neural tissue engineering applications. In conclusion, we highlight the cutting-edge potential of biohybrid neural interfaces as a novel class of materials with an immense prospect in the realm of neural interface engineering. Collectively, these insights pave the way for innovative therapeutic strategies and tissue engineering-based approaches to treating sensorineural hearing loss SNHL.

Major causes of spiral ganglion neuron loss

Noise exposure

Noise is a common cause of SNHL, which is mainly characterized by decreased auditory sensitivity and increased hearing threshold, manifested as temporary threshold shift (TTS) or permanent threshold shift (PTS). Hearing threshold shift is mainly correlated with noise intensity, frequency, and exposure time [9]. Moderate TTS is a manifestation of protective purinergic hearing adaptation and is characterized by reversible noise-induced damage to stereocilia or synapses [10]. PTS represents permanent damage to HCs and synapses, which can be confirmed by the loss of IHC ribbon synapses, swelling of afferent terminals of SGNs, and secondary or primary neuronal degeneration [11–14]. Exposing mice to moderately wide frequency noise for 4 consecutive weeks led to an increase in the expression of their SGN purinergic receptors, mediating enhanced medial olivocochlear reflex [15]. High-intensity noise exposure (>100 dB sound pressure level, SPL) or repetitive overstimulation can induce demyelinating lesions in SGNs, and the effects of such injuries may be delayed for months or years [16]. Surprisingly, even

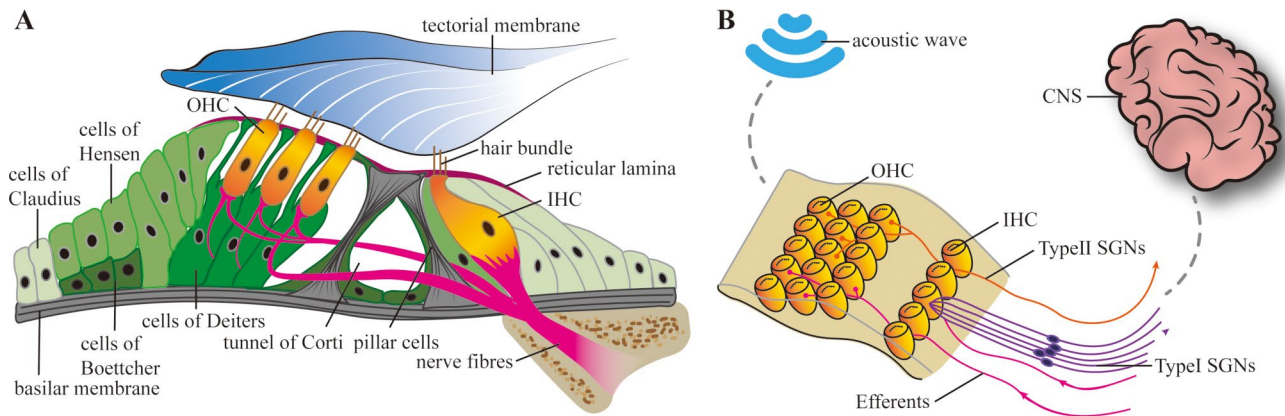


Fig. 1 (A) The organ of Corti. (B) A schematic diagram illustrating the transmission of sound from the sensory epithelium to the auditory central nervous system, highlighting the subtypes of the SGN

mild auditory trauma associated with TTS results in more than 50% loss of synapses between cochlear nerve fibers and IHCs, but no loss of HCs and no change in hearing threshold [17]. This form of impairment is considered to be one of the major causes of hidden hearing loss, which causes patients to lose speech recognition and music appreciation in complex sound environments [18]. Noise exposure-induced SGN degeneration varies according to its corresponding cochlear topology, with a significantly higher incidence in the base than the apex. Correspondingly, SGN degeneration leads to reduced cochlear nerve nuclear volume, apoptosis, and smaller nuclei [19].

Increased oxidative stress, excessive release of excitatory neurotransmitters, altered ionic environment, and decreased blood flow are all potential mechanisms of noise-induced hearing loss (NIHL) [20, 21]. In addition, there is increasing evidence that cochlear inflammation also appears to be involved. The cochlea has previously been described as an immune-privileged organ that protects the auditory system from immune system responses. Resident macrophages in the mammalian cochlea have been continuously found to be distributed in areas of spiral ligament, SGN, stria vascularis, basilar membrane, and bone spiral lamina, suggesting the innate immunity of the cochlea [22–24]. The number of activated macrophages in the basal curvature of the basilar membrane, spiral ligament, and spiral limbus regions increases significantly under noise exposure [25, 26]. Like microglia, activated cochlear macrophages also undergo morphological changes. Following acoustic trauma, cochlear macrophage protrusions become shorter and change their shape into an amoeboid phenotype, with increased proliferation and phagocytosis [27, 28]. Compared to TTS, mice with PTS have more severe damage to the stria vascular and spiral ligament fibrocytes, and significantly higher levels of cochlear proinflammatory cytokine (such as IL-1 β , IL-6, and TNF- α) expression [29]. TNF- α was found to markedly elevate the generation of nitric oxide (NO) and the

expression of inducible nitric oxide synthase (iNOS). The inhibition of iNOS significantly mitigated TNF- α -induced HC death and caspase-3 activation [30]. Moreover, TNF- α treatment augmented the expression of phosphorylated p38 and phosphorylated ERK, which contributed to the amplification of the pathological response [30–32]. Noise-induced intracochlear IL-6 expression has also been shown to be detrimental, leading to excessive and prolonged inflammatory responses. IL-6 blockers, tocilizumab, MR16-1, etc., have shown therapeutic effects on NIHL by suppressing neuronal loss [33, 34]. IL-1 β activation in macrophages contributes to noise-induced hearing loss, but inhibition doesn't reverse damage [35].

Aging

Age-related loss of HCs and SGNs is a major contributor to age-related hearing loss (ARHL), which affects half of the population older than 75 years [4]. Early ARHL manifests as reduced speech recognition and impaired frequency resolution in noisy environments [36]. Currently, effective clinical treatment options for ARHL are limited to medical devices such as hearing aids and CIs, but they are premised on the presence of sufficient numbers of SGN [37]. ARHL is often accompanied by accelerated loss of SGN due to genetic and environmental interactions, and advanced stages of SGN degeneration may occur independently of age-related HC loss. The major pathways involved include the IGF-1 pathway and the lipophilic/steroid hormone pathway [4]. Studies have shown that CBA / CAJ mice with early noise exposure are more susceptible to ARHL [38]. But even in the absence of noise exposure throughout life, CBA / CAJ mice exhibited ARHL and the loss of cochlear synapses and nerves can be observed simultaneously [39]. Wong et al. suggested that damage and accumulation of various etiologic factors combine to cause ARHL [40]. Improving cochlear defense against oxidative stress through antioxidant intervention is a reasonable and feasible approach to

prevent ARHL. Increased mitochondrial DNA mutations in patients with ARHL may lead to accelerated cellular damage by reactive oxygen species (ROS) [41]. Copper/zinc superoxide dismutase is the first line of defense against ROS-induced oxidative damage, and its absence leads to significant loss of SGNs in mice [42]. Mitochondrial damage also affects apoptosis and cellular calcium signaling. Calcium dysregulation is a critical factor in the pathogenesis of age-related hearing loss and SGN degeneration. Nuclear factor kappa B (NF- κ B), a key regulator of calcium signaling, is essential for preventing excitotoxic damage and neural degeneration in the cochlea. The absence of NF- κ B activity leads to disrupted calcium homeostasis, exacerbating age-related hearing loss and SGN degeneration without hair cell loss or endocochlear potential decline [43]. Furthermore, NF- κ B knockout mice also showed increased sensitivity to low-level noise exposure. It is generally accepted that age-related neuronal death modalities mainly result from apoptosis [44, 45]. However, in the auditory cortex, iron deposition and ferroptosis may also contribute to presbycusis, characterized by speech comprehension and speech localization difficulties [46]. Changes in the expression of excitatory and inhibitory transmitters and receptors in auditory central neurons with age can also lead to changes in sound perception and affect signal processing [47].

Ototoxic drugs

More than 150 ototoxic drugs have been discovered, the most common of which include aminoglycoside antibiotics (AABs), loop diuretics, and antitumor drugs (e.g., cisplatin) [48]. AABs, such as Tobramycin, Gentamicin, Amikacin, and Neomycin, are known to induce ototoxicity, which compromises inner ear cell function. It is widely accepted that AABs exhibit toxic effects on SGNs, primarily manifested as auditory and vestibular disorders. This is due to the damage caused by AABs to the hair cells in the inner ear, which are responsible for detecting sound and maintaining balance. These antibiotics penetrate the blood-cochlear barrier and blood-brain barrier, accumulating within the inner ear, particularly in sensory hair cells and supporting cells. Their mechanisms of action include direct damage to hair cells by interfering with their metabolism, leading to cell death and subsequent hearing loss. Additionally, AABs inhibit key enzyme activities, such as adenosine acidase and ribosomal S12 protein, which can affect cellular energy metabolism and protein synthesis, resulting in cell damage. Furthermore, these antibiotics may cause abnormal increases in intracellular calcium ion concentrations, triggering apoptosis and damaging hair cells. Lastly, AABs can impact cell membrane stability, disturbing the balance of substances across the membrane and further cell damage [48–50]. Cisplatin produces ototoxicity

primarily through inhibition of the cochlear antioxidant defense system and activation of the apoptotic pathway in the inner ear, involving 62% of adult patients and 60% of pediatric patients [51]. Specifically, cisplatin is highly active on thiol-containing molecules such as glutathione, and its depletion leads to the accumulation of ROS [52], which in turn leads to the activation of apoptosis [53]. After cisplatin exposure, autophagic flux in SGNs is activated to counteract cisplatin-induced oxidative stress [54]. Febles et al. found that the combined use of antioxidants, p53 inhibitors, and neurotrophic factors (NTFs) was otoprotective against cisplatin exposure [51]. Given the risk of ototoxicity, especially with long-term or high-dose usage, it is crucial for healthcare professionals to carefully assess patient conditions and risk factors when prescribing these medications, and to implement necessary measures to monitor and prevent ototoxicity.

Infections

Many viruses can cause SNHL, and cytomegalovirus (CMV) is a major source of infection leading to congenital deafness [55]. Systematic evaluation and meta-analysis based on clinical data showed that CMV-positive fetuses tested by amniocentesis had a 22% probability of exhibiting severe SNHL at birth [56]. Additionally, in a mouse model of CMV infection, Sung et al. found that peripheral CMV infection resulted in focal cochlear infection and inflammation. Moreover, 50–60% of mice had significantly elevated auditory brainstem response (ABR) thresholds and reduced numbers of SGNs, synapses, and neurites, but the number and morphology of hair cells in the organ of Corti were preserved. CMV-induced SGN death may result from the simultaneous activation of the p53/JNK and NLRP3/caspase-1 signaling pathways leading to apoptosis and pyroptosis [57, 58]. Wu et al. found that inhibition of inflammasome assembly by blocking apoptosis-associated speck-like protein containing caspase recruitment domains could inhibit the activation of caspase-1, thereby rescuing the death of SGNs and ameliorating hearing loss in CMV-infected mice [59]. Additionally, Rubella, Lymphocytic choriomeningitis virus (LCMV), human immunodeficiency virus (HIV), herpes simplex virus (HSV), and other various viruses can also induce congenital or acquired deafness [55]. According to clinical case analysis, the 2019 coronavirus disease (COVID-19) infection is also a high-risk factor for SNHL [60]. The results of both transient evoked otoacoustic emissions and high-frequency pure-tone threshold amplitudes demonstrated a substantially higher likelihood of hearing impairment in COVID-19-positive patients [61]. Although steroid therapy is the standard means to improve SNHL induced by COVID-19 infection, less than half of patients benefit from treatment [62].

Genetic factors

Hereditary hearing loss is also the most common type of congenital deafness, accounting for approximately 50% of congenital hearing loss [63–66]. In addition, genetic factors are also associated with some late-onset hearing loss. Patterns of inheritance of hearing loss-associated genetic variants typically include autosomal dominant, autosomal recessive, or X-linked [67]. Currently, more than 120 non-syndromic hearing loss genes have been identified (<https://hereditaryhearingloss.org>). Among these, mutations in genes associated with neuronal survival and synaptic transmission are more likely to cause primary SGN degeneration, such as POU class 3 homeobox 4 (POU3F4) and soluble carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 8 (SLC17A8) [68]. POU3F4 is widely expressed in the otic mesenchyme, which is essential for axon bundling of SGN development. Disrupted formation of SGN forming radial bundles and synapses causes malformations of middle and inner ear development when POU3F4 is deleted in the otic mesenchyme [69]. Postnatally, POU3F4 knockdown also leads to apoptosis of SGNs, but does not appear to result in changes in the proportion of SGN isoforms [70]. SLC17A8 encodes vesicular glutamate transporter-3 (VGLUT3), which is responsible for the accumulation of glutamate in the synaptic vesicles in cochlear hair cells and the release of glutamate to auditory nerve middle terminal receptors. It has been shown that SLC17A8 exon 2 deletion mice lack auditory nerve reflexes to auditory stimuli, but have detectable ABR evoked by dotclick stimuli and otoacoustic emissions [71]. Variants in genes associated with HC and supporting cell (SC) degeneration predispose to secondary degeneration of SGNs. Gap junction protein, beta 2 (GJB2) expressed in SCs is a commonly mutated gene causing mild to severe nonsyndromic deafness and is involved in transcellular signaling, metabolic supply, and fluid homeostasis [72]. The Otoferlin (OTOF) gene mutation leads to a reduction and apoptosis of SGNs, which may be a secondary defect due to the lack of otoferlin in IHCs. Glutamatergic synaptic inputs are important for the survival of SGNs [73]. Study also shows that hypothyroidism causes a defect in the pruning of afferent type II SGNs and delays the maturation of OTOF expression [74]. However, there is currently no effective pharmacological treatment for congenital deafness caused by OTOF mutations. An ongoing clinical trial is investigating the safety and efficacy of gene therapy using an AAV serotype 1 carrying a human OTOF transgene (AAV1-hOTOF) for children with autosomal recessive deafness 9. Preliminary results show that AAV1-hOTOF gene therapy is safe and effective in improving hearing and speech perception in these children [75]. Additionally, mutations in other genes, including antioxidant defense

and mitochondria-related genes, can also render humans vulnerable to other deafness factors, which can further exacerbate SGN damage [76].

Three elements of tissue engineering strategies to preserve and regenerate spiral ganglion neurons

The concept of tissue engineering was first proposed by the National Science Foundation in 1987 and was also known as "regenerative medicine", which refers to the utilization of bioactive substances to reconstruct or repair organs and tissues through bioengineering techniques. At present, tissue engineering has made great progress in many fields, for example, tissue-engineered skin and cornea have been used in clinical applications. The core of tissue engineering is to establish a three-dimensional spatial complex of cells and biomaterials, which mainly involves three elements: seed cells, scaffold materials, and growth factors.

Seed cells

The challenges of regenerative strategies for auditory neurons include not only the survival and migration of transplanted stem cells to functional regions but also their functional integration with the host. Therefore, the selection of appropriate candidate cells is crucial. Taking stem cells as seed cells to participate in SGN regeneration is an important strategy, and commonly used stem cells mainly include resident stem cells of the organ of Corti as well as other exogenous stem cells. Multipotent inner ear stem cells with self-renewal capacity, expressing marker genes for the developing inner ear and nervous system, were first identified in the adult mouse utricular sensory epithelium in 2003 by Li et al. [77]. Additionally, the existence of SGN-neural stem cells (NSCs) with self-renewal ability and potential to differentiate into different subtypes of SGN has offered new hope for SGN regeneration [78]. Unfortunately, the number of SGN-NSCs dramatically decreases with age [79]. Senn et al. also concluded that the number and limited proliferative capacity of stem cells in the adult cochlea are insufficient to differentiate into inner ear cell types [80]. Therefore, exogenous stem cells seem to be the preferable seed cell source for SGN regeneration, including embryonic stem cells (ESCs), NSCs, induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), and others.

ESCs are multipotent, self-renewing cells derived from the developing blastocyst [81]. As early as 2003, investigators were able to differentiate mouse ESCs into inner ear progenitors carrying genes related to inner ear development [82]. As illustrated in Fig. 2A(i), the use of human embryonic stem cell (hESCs) otic progenitors to generate SGNs was first reported by Chen et al. (2012). More critically, this study also showed that these SGNs generated from stem cells could be injected into an

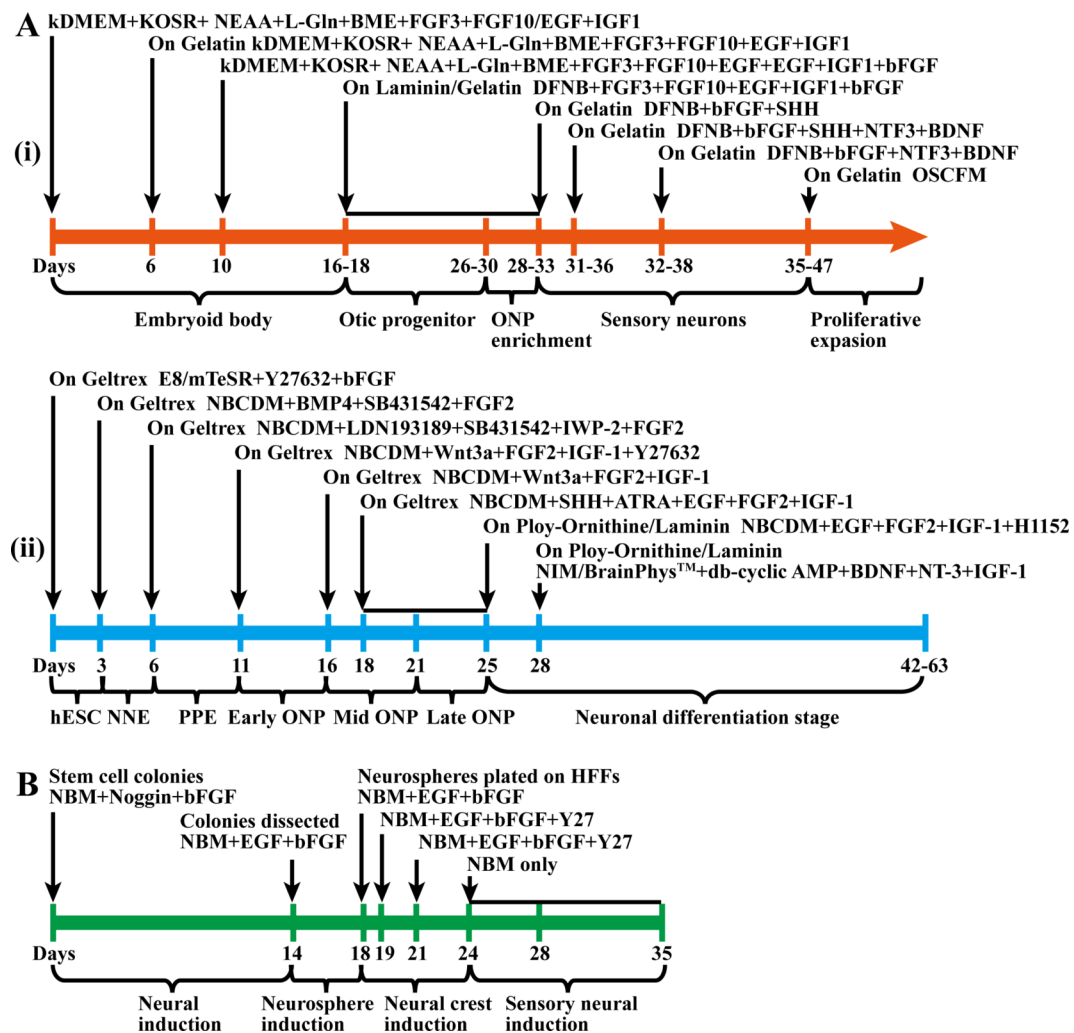


Fig. 2 (A) Schematic summary of two protocols and schedules for generating late-stage ONPs from undifferentiated human ESCs. (B) Protocol for neurosensory lineage from hiPSCs. kDMEM, Knockout Dulbecco's modified Eagle's medium; KOSR, Knockout serum replacement; NEAA, Nonessential amino acids; L-Gln, L-Glutamine; BME, β -mercaptoethanol; FGF3, Fibroblast growth factor 3; FGF10, Fibroblast growth factor 10; EGF, Epidermal growth factor; IGF1, Insulin-like Growth Factor 1; bFGF, Basic fibroblast growth factor; DFNB, Dulbecco's Modified Eagle's Medium (DMEM) with Ham's F12 and N2/B27; SHH, Sonic hedgehog; NTF3, Neurturin; BDNF, Brain-derived neurotrophic factor; OSCFM, otic stem cell full media (DFNB plus 20 ng/ml bFGF, 50 ng/ml IGF1 and 20 ng/ml EGF); CDM, Chemically defined medium; NBCDM, CDM plus 1% N2 and 2% B27; BMP4, Bone Morphogenetic Protein 4; FGF2, Fibroblast growth factor 2; IWP-2, Insulin-like growth factor binding protein 2; Wnt-3a, Wingless-type MMTV integration site family member 3a; ATRA, All-trans retinoic acid; NNE, nonneuronal ectoderm; PPE, preplacodal ectoderm; ONP, Otic neuron progenitor; OEP, Otic epithelial progenitor; HFF, Human foreskin fibroblasts; NBM, Neurobasal A with 1% N2, 2% B27, 2 mM L-Gln and 0.5% penicillin/streptomycin; Noggin, NOGGIN neuralgenic cell-specific growth inhibitor

auditory neuropathy model, where native SGNs had been destroyed by ouabain administration, and were incorporated into the spiral ganglion to produce a degree of functional restoration of hearing. This paves the way for a new auditory regenerative strategy [83]. Subsequently, Matsuoka et al. refined the protocol for inducing step-by-step differentiation of hESCs into SGN-like cells based on the key processes and signaling pathways of SGN lineage development, as depicted in Fig. 2A(ii). The refined steps followed the developmental order inherent in hESC differentiation into SGNs, in descending order from non-neuronal ectoderm, preplacodal ectoderm, early

prosensory ONPs, late ONPs, and cells with cellular and molecular characteristics of human SGNs. The optimized differentiation protocol seemed to increase the proportion of the target cell population.

Generating functional neurons from ESCs capable of accurately encoding and transmitting sound information to the brainstem is no easy task. Matsuoka et al. co-cultured SGNs with brainstem slices and only one-fifth of the cells responded to electrical stimulation [84]. Despite subsequent research efforts by other groups, such as attempts to induce differentiation using a three-dimensional culture system [85], there remained limited

progress in the functional maturation of the induced SGN-like cells.

Glutamatergic primary auditory neurons possess a large number of ion channels, a key feature of which is the ability to reliably track high-frequency stimuli [86]. Needham et al. found that sensory neurons derived from hESCs, although reliably activated by stimulation with a train of 20 pulses per second (pps), were markedly different from auditory neurons when subjected to high-frequency stimulation at 50 pps in mammals [87]. This suggests that manipulation of *in vitro* differentiation alone may not enable terminal differentiation of auditory neurons. Moreover, spontaneous activity and electrical stimulation can promote mature electrophysiological phenotype in auditory neurons [88].

Adult NSCs can also be used to replace SGNs, mainly NSCs derived from the hippocampus and olfactory mucosa. It was shown that the formation of new ribbon synapses was observed by co-culturing neurons derived from SD rat embryos (E12-E14) NSCs with P3 rat cochlear sensory epithelial explants [89]. Zhang et al. demonstrated that the migration of olfactory NSCs into the cochlea via the transplantation route through the lateral wall of the cochlea may proceed through gaps present in the basement membrane and eventually differentiate into SGNs [90]. Xu et al. highlighted olfactory NSCs' ability to secrete NTFs to protect SGNs from injury, including nerve growth factor (NGF) and neurotrophin 3 (NT-3) [91]. As early as 2005, Regala et al. found that several types have the potential to reconstruct the neuronal conduit after neuronal injury between the inner ear and the central nervous system by implanting ESCs, adult NSCs, and fetal dorsal root ganglia into the injured vestibulocochlear nerve of adult rats and guinea pigs [92]. In the same year, a study from the Hearing and Communication Research Centre at Karolinska Institutet also raised the possibility that embryo DRG xenotransplantation may offer therapeutic possibilities for patients with profound deafness [93]. However, there are fewer sources of cell donors as mentioned above.

Takahashi et al. reprogrammed adult fibroblasts to an embryonic-like state, termed iPSCs, by introducing POU class 5 homeobox 1 (Oct3/4), SRY-box 2 (Sox2), myelocytomatosis oncogene (*c-Myc*), and Kruppel family member 4 (*Klf4*) into adult fibroblasts [94]. Among them, Sox2 is one of the earliest markers for the development of inner ear sensory domains and is required for the development of mammalian inner ear sensory organs [95]. Sox2 can upregulate neurogenin 1 (*Neurog1*) expression in otic progenitors and mediates neuronal differentiation in cooperation with the key transcription factors Eyes absent 1 (*Eya1*) and SIX homeobox 1 (*Six1*) that initiate the neuronal developmental program [96]. Gunewardene et al. differentiated two hiPSC cell lines into neurosensory

lineage *in vitro* by a combination of signaling molecules including basic fibroblast growth factors (bFGFs), bone morphogenetic proteins, sonic hedgehog, Y27632 and NTFs (brain-derived neurotrophic factor, BDNF and NT3) (Fig. 2B). The induced neurons expressed ganglion neuronal markers (*NeuroD1*, *Brn3a*, *Islet1*, β III tubulin, Neurofilament kDa 160) and were electrically active. In addition to ganglion neuronal markers, hiPSCs-derived neurosensory progenitors expressed dorsal hindbrain marker (*Pax7*), otic placode marker (*Pax2*), preneural sensory marker (*Sox2*), and sensory auditory neuron markers (*GATA* binding protein 3, *GATA3* and vesicular glutamate transporter 1, *VGLUT1*). Unfortunately, differentiation into the desired lineage using current induction protocols is less consistent, with differences in the expression levels of these markers between hiPSCs lines and even within the same cell line [97]. Meanwhile, teratoma formation is also a nonnegligible safety concern for iPSC transplantation. Zhu et al. indicated that teratomas formed from undifferentiated iPSCs were observed in the cochlea after transplantation of iPSCs through the round window membrane route [98].

MSCs face fewer ethical controversies compared to ESCs because MSCs are derived from adult sources such as bone marrow or adipose tissue, avoiding the ethical issues associated with the destruction of embryos. Additionally, MSCs have shown potential in various therapeutic applications without the risk of immune rejection or ethical concerns related to pluripotency. Moreover, MSCs have been favored by researchers for their long-term proliferative capacity *in vitro*, multilineage differentiation potential, and immunomodulatory properties. Clinical outcomes of autologous MSC transplantation in patients for the treatment of SNHL demonstrated its safety but did not achieve the desired therapeutic effects [99]. This result may be attributed to the method of transplantation by intravenous injection. In animal experiments, local injection is mostly used for bone marrow stromal cell (BMSC) transplantation [100–103]. It has been shown that injection of BMSCs into guinea pig scala tympani with autoimmune SNHL significantly reduced ABR wave III thresholds [102]. Sujeong et al. used basic fibroblast growth factor and forskolin for neural induction of hMSCs isolated from bone marrow obtained during mastoidectomy. Neural-induced human MSCs (Ni-hMSCs) exhibited high levels of expression of neuronal markers and ion channel markers, as well as voltage-dependent sodium currents. Moreover, Ni-hMSCs transplanted into the scala tympani of guinea pigs successfully migrated into the spiral ganglia and expressed the neuron-specific marker NeuN, which was compensatory for neomycin-induced SGN deficiency [101]. Kamiya et al. also showed good hearing restoration in a rat model of acute SNHL induced by mitochondrial toxin through lateral wall

transplantation of BMSCs [103]. Therefore, BMSCs have the potential to serve as seed cells for both differentiation into SGNs and maintenance of inner ear homeostasis. However, there are still some inherent limitations to the source of BMSCs, such as a painful isolation process and a decrease in stemness and expansion rates as the donor ages [104].

Fetal adnexa-derived MSCs such as umbilical cord blood, placenta, amniotic fluid, and Wharton's jelly MSCs (WJ MSCs) are easy to isolate from discarded tissues after birth. With low immunogenicity, stronger stemness than adult MSCs, and no ethical concerns, these MSCs have great potential in regenerative medicine [105, 106]. A clinical study involving 30 children with sensorineural hearing with mucopolysaccharidosis demonstrated that the patients' ABR click threshold increased by an average of 19 dB after hematopoietic stem cell transplantation [107]. In another study, researchers transplanted BDNF, glial cell line-derived neurotrophic factor (GDNF), and NT3-treated human placental MSC into neomycin- and ouabain-induced SNHL guinea pigs and found improved ABR thresholds and distortion product otoacoustic emission (DPOAE) levels, as well as an increase in SGN number in all turns of the cochlea [108]. Amniotic fluid MSCs have also been described as an alternative to SGNs after injury [109]. WJ MSCs isolated from umbilical cords are negative for MHC class-II and do not produce graft rejection or tumorigenicity *in vivo*, providing a good candidate cell for both allogeneic and xenogeneic transplantations [110]. Xu et al. found that tracers labeling WJ MSCs were detected in the cochlear blood vessels, bony wall of scala tympani, and spinal ganglion nerve fibers, which could rescue noise-induced SNHL in minipigs [111]. Although Devarajan et al. successfully differentiated human umbilical cord MSCs into hair cell-like cells by overexpressing Atonal Homolog 1 (Atoh1) [107], further study of whether WJ MSCs can be induced to differentiate into the SGN phenotype has not yet been reported, and further exploration is needed.

Other MSC seed cell candidates mainly include dental pulp stem cells, adipose stem cells, and human limbal MSCs (HL-MSCs). Among them, human dental pulp stem cells (DPSCs) and stem cells from human exfoliated deciduous teeth are derived from the neural crest during development with high potential for neuronal differentiation. The neural precursor cells generated from them could differentiate into SGNs highly expressing tyrosine kinase receptor B (TrkB), GATA3, and SYP with exhibiting intracellular calcium oscillations by co-culturing with auditory brainstem slices containing cochlear nuclei [112]. Similar results were observed in Sprague Dawley rats with SNHL [113]. Autologous adipose tissue-derived stem cells were reported to restore hearing in a 19-year-old Caucasian female with autoimmune hearing

loss [114]. Animal studies have shown that human adipose tissue-derived stem cells induced with forskolin and bFGF could migrate to the spiral ganglion and support the repair of damaged SGNs, thus contributing to the recovery of SNHL [115]. HL MSCs also displayed significant protection against ouabain-induced SGN loss in mouse models. Their ABR results suggested a significant improvement in hearing after three months of HL MSC transplantation [116].

Despite significant advancements in the differentiation of seed cells into SGNs, researchers still face numerous challenges (Table 1). Firstly, the efficiency and predictability of stem cell differentiation need to be improved, as the results exhibit considerable variability, which limits their clinical applicability. Furthermore, the differentiated cell populations often contain various cell types rather than solely SGNs, potentially affecting cell purity and functionality. Immunological rejection and safety issues should not be overlooked, as allogeneic stem cell-derived products may elicit immune responses and carry the risk of long-term toxicity or carcinogenicity. Additionally, the use of ESCs and iPSCs is surrounded by ethical and legal controversies and restrictions. Challenges in large-scale production and application include optimizing production processes, controlling costs, and ensuring quality assurance. Individual variations and disease states also pose extra challenges for cell therapy, as cells from different individuals and disease states may exhibit different differentiation potentials and responsiveness. Lastly, the long-term stability and functional efficacy of differentiated SGNs *in vivo* need to be monitored to ensure durable and safe therapeutic outcomes. Despite these challenges, the research achievements in the differentiation of seed cells into SGNs are notable. With the continuous advancement of scientific technology, it is believed that these challenges will be gradually overcome, offering new prospects for the treatment of neurodegenerative diseases.

Biomolecules

NTFs are proteins produced and secreted by neurons, glial cells, sensory cells, and muscle fibers and can be involved in regulating the survival, proliferation, axonal and dendritic growth, and patterning of cochlear neurons. Moreover, NTFs are also key biomolecules in the regulation of the expression and activity of important functional proteins (e.g., ion channels and neurotransmitter receptors). The four common NTFs in mammals, BDNF, NT-3, NGF, and neurotrophin-4/5 (NT-4/5), all bind to p75 neurotrophin receptor (p75^{NTR}) and Tyrosine receptor kinase (Trk); specifically, NGF binds to TrkA, BDNF and NT-4/5 bind to TrkB, and NT-3 binds to tyrosine kinase receptor C (TrkC) [117]. Trk receptors are a family of tyrosine kinases that regulate synaptic

Table 1 Types of seed cells

Cell type	Source	Key advantages	Key challenges	Example papers
SGN-neural stem cell	Cochlea	<ul style="list-style-type: none"> • Self-renewal • Endogenous repair 	<ul style="list-style-type: none"> • Insufficient quantity • Decreases with age • Limited proliferative capacity • Unstable differentiation efficiency and direction 	[77-80]
Neural stem cell	Adult brain	<ul style="list-style-type: none"> • Self-renewal • Endogenous repair 	<ul style="list-style-type: none"> • Limited sources • Unstable differentiation efficiency and direction • Low functional maturation • Allogeneic transplant immunoreaction 	[89, 92]
Embryonic stem cell	Embryo	<ul style="list-style-type: none"> • High functional maturity 	<ul style="list-style-type: none"> • Limited sources • Immune rejection • Oncogenic risk • Ethical controversies • Difficulty in differentiation control • Unstable differentiation efficiency and direction 	[82-85, 87, 92]
Induced pluripotent stem cell	Adult cell	<ul style="list-style-type: none"> • Relatively controllable differentiation process • Low ethical controversy • Low immune rejection • Personalized medicine 	<ul style="list-style-type: none"> • Genetic mutation • Epigenetic changes • Oncogenic risk • Unstable differentiation efficiency and direction • Low functional maturation • Transplant challenges 	[97, 98]
Olfactory ensheathing cell	Olfactory system	<ul style="list-style-type: none"> • High regenerative capacity • Low immune rejection • Low ethical controversy 	<ul style="list-style-type: none"> • Limited sources • Unstable differentiation efficiency and direction • Low functional maturation • Transplant challenges 	[90, 91]
Dorsal root ganglion	Embryo	<ul style="list-style-type: none"> • High functional maturity 	<ul style="list-style-type: none"> • Limited sources • Ethical controversies • Infection risk • Oncogenic risk 	[92, 93]
Bone marrow-derived mesenchymal stem cell	Bone marrow	<ul style="list-style-type: none"> • Wide source availability • Self-renewal • Easy to obtain • Low ethical controversy 	<ul style="list-style-type: none"> • Immune rejection • Stemness decreases with age • Unstable differentiation efficiency and direction • Harvesting Pain • Infection risk 	[99-104]
Umbilical cord blood stem cell	Umbilical cord blood	<ul style="list-style-type: none"> • High proliferation capacity • Immunomodulation 	<ul style="list-style-type: none"> • Complex harvesting and separation procedures • Stringent storage and transportation conditions 	[106, 107]
Wharton's jelly mesenchymal stem cell	Umbilical cord tissue	<ul style="list-style-type: none"> • Wide source availability • Immunomodulation • Low ethical controversy 	<ul style="list-style-type: none"> • Unstable differentiation efficiency and direction • Long-term Safety • Low functional maturation • Transplant challenges 	[105-107, 110, 111]
Dental pulp stem cell	Dental pulp	<ul style="list-style-type: none"> • High proliferation capacity • Wide source availability • Easy to obtain 	<ul style="list-style-type: none"> • Complex Separation Procedures • Harvesting Pain • Unstable differentiation efficiency and direction 	[112, 113]
Adipose-derived stem cell	Adipocyte	<ul style="list-style-type: none"> • Wide source availability • Self-renewal • Easy to obtain • Low ethical controversy 	<ul style="list-style-type: none"> • Complex Separation Procedures • Harvesting Pain • Unstable differentiation efficiency and direction 	[114, 115]

strength and plasticity in the mammalian nervous system [118]. Tyrosine phosphorylation sites in the cytoplasmic domain of Trk receptor recruit intermediate in intracellular signaling cascades that activate several small G proteins, as well as pathways regulated by MAP kinase, PI 3-kinase and phospholipase-c-gamma (PLC-gamma) [118]. The activation of $p75^{NTR}$ leads to the activation of signaling pathways such as NF- κ B and Jun kinase [119]. In some cases, $p75^{NTR}$ synergizes with Trk receptor-activated signaling pathways, and in other cases, $p75^{NTR}$ antagonizes Trk receptor-activated signaling pathways. It

has been shown that any of these four classes of NTFs can induce apoptosis by activating $p75^{NTR}$, but this effect is blocked by Trk receptor activation [120]. In addition, the conformation of Trk receptors is also affected by $p75^{NTR}$, leading to changes in the specificity and affinity of ligand binding [118]. In adult neurons, NTFs are required for their persistence, and they enhance transmitter release in neurons expressing the respective signal transducing Trk receptors, which act as selective retrograde messengers to modulate synaptic potency [121]. Co-activation of $p75^{NTR}$ and Trk may result in cell proliferation, synaptic

growth, or specific differentiation depending on the specific NTFs and the formation of coreceptor complexes [122].

BDNF is one of the most studied protective NTFs against SGN injury, with protective effects against noise, inner ear ischemic, and pharmacological SGN injury, and is also a common factor for inducing neuronal differentiation [123]. BDNF mRNA was found in hair cells of the rat ear in early development, but not in the adult organ of Corti [124]. In contrast, TrkB mRNA is still strongly expressed in spiral and vestibular ganglia in adulthood [125]. Calcium signaling plays a key role in signal transduction of apoptosis. When noise and ototoxic drugs cause SGN damage, Ca^{2+} influx is exacerbated and the intracellular Ca^{2+} concentrations increase, thereby initiating the apoptotic program [126]. BDNF can protect neurons from damage by regulating the expression of T-type calcium channel protein to affect Ca^{2+} influx [127]. In addition to its anti-apoptotic effects, BDNF has antioxidant properties [128, 129]. Neuronal damage is directly related to the excessive release of excitatory amino acids (glutamate), in part due to the massive production of oxygen-free radicals induced by intracellular Ca^{2+} overload [130]. An in vitro study by Wille et al. revealed that loading BDNF with heparan sulfate-coated fibers prolonged the release of NTFs for up to 110 days and greatly improved SGN survival [131]. Additively, BDNF has also exhibited its therapeutic potential for long-term deafness. Miller et al., treated aminoglycoside-induced deafness by continuous infusion of BDNF and FGF1 into the scala tympani of guinea pigs. It was found that the combination of BDNF and FGF1 significantly protected SGNs after 4 or 8 weeks of aminoglycoside treatment, although the effect at 8 weeks appeared to be relatively weak [132]. Moreover, genetically modified MSCs can also achieve long-term administration of BDNF for SGN protection [133].

NT-3 is the most abundant NTF in early development. Its deletion will cause severe loss of sensory nerves in mice, and expression of NT-3 and TrkC can be observed as early as before neural tube closure [134]. During development, NT-3 is continuously expressed in inner and outer hair cells, but only in IHCs throughout adulthood, with higher levels in the apical than basal regions. TrkC mRNA is expressed in the spiral and vestibular ganglia, but is weaker than TrkB around birth and in adulthood [125]. Thus, NT-3 has a wide range of biological activities that not only regulate neuronal differentiation but also promote the survival and repair of injured neurons [135, 136]. Wan et al. showed that NT-3 is required for the formation and maintenance of the ribbon synapses in the hair cells of the cochlear epithelium in the post-natal inner ear. Mice overexpressing NT-3 recovered more quickly after noise trauma [137]. Loss of target

innervation caused by aminoglycoside toxicity and substantial degeneration of adult SGNs can be prevented by infusion of NT-3 into the guinea pig membranous labyrinth [138]. Considering the significant obstacles and trauma risks posed by exogenous NT-3 protein delivery, 1Aa and a covalent conjugate of 1Aa with risedronate, Ria-1Aa, small molecules that mimic NT-3 function and act on TrkC receptors, were designed and synthesized. Both can diffuse through the round window membrane and enrich in the lymph, stimulate SGNs, and promote the regeneration of synapses between SGNs and inner hair cells, balancing the surgical risks brought by labyrinthotomy [139].

Currently, there are not many studies on NT-4/5 in neuroprotection, but a study in amyotrophic lateral sclerosis has shown that despite the expression of all four categories of NTFs mentioned above [140], it seems that only exogenous NT-4/5 application has therapeutic effects, while BDNF and NT-3 did not show neuroprotective effects [141]. A study by Zheng et al. suggested that NT-4/5 promoted rat SGN survival in vitro with potency comparable to that of BDNF and stronger than NT-3. Furthermore, NT-4/5 protected SGNs from the neurotoxic effects of the anticancer drug cisplatin [142]. These results indicate the potential of NT-4/5 in the prevention and treatment of hearing impairment caused by primary auditory neuron damage.

NGF is also one of the most studied neurotrophic molecules to date. NGF plays an important role in the establishment and refinement of neuronal connectivity during neuronal development and regeneration. It can provide trophic support to neurons through axonal retrograde transport patterns [143], stimulate growth cone migration and sprouting of axon lateral branches [144, 145], and promote axonal regeneration (including myelinated and unmyelinated axons) [146]. It has been shown that in DBA/2J mice with early-onset progressive hearing loss, long-term intramuscular administration of NGF from birth decreased their ABR thresholds and increased DPOAE amplitudes [147]. Hearing improvement was observed after implanting olfactory epithelial neural stem cells (oe NSCs) into the cochlea of rats with noise-induced hearing loss through a retroauricular approach. The authors suggested that this may be due to the migration of oe NSCs into Rosenthal's canal and the secretion of NGF and NT-3 to prevent SGN injury [91]. In experiments using DRG to replace degenerating SGNs to rescue hearing loss, the addition of NGF effectively promoted the penetration of DRG neurites to osseous modiolus and projection to SGNs [93]. Pan neurotrophin 1 (pnt-1) is a synthetic trophic factor combines the active domains of the neurotrophins NGE, BDNF, and NT-3, which has potential applications in nerve injury repair and regeneration [148]. However, whether it can exert

trophic support and regenerative effects on the SGNs in auditory nerve injury has not been reported.

The FGF homologous factor subfamily consists of 18 secreted proteins and 4 intracellular proteins. Its primary role is to protect the neurovascular unit and facilitate neurofunctional repair by activating signaling pathways like PI3K-Akt and peroxisome proliferator-activated receptors (PPAR γ). Moreover, it inhibits NF- κ B-mediated inflammatory responses, oxidative stress, neuronal apoptosis, and regulating neuronal differentiation and excitability [149–151]. The combination of fibroblast growth factor 2 (FGF2) and GDNF supports the long-term survival of SGNs and prevents secondary neuronal degeneration [152]. This may be attributed to the ability of the FGF family to strongly enhance neuronal neurite outgrowth from SGNs [152, 153] and extend to the sensory epithelium of the transfectants [154], consequently promoting the generation of ribbon synapses [155]. In vitro studies have demonstrated that the combination of FGF2 and retinoic acid (RA) induces the differentiation of otic cell lineages derived from hPSCs into otic placodal cells [156]. In addition, by integrating FGF2 with IGF and epidermal growth factor (EGF) within a three-dimensional scaffold, human gingival MSCs can be induced to differentiate into audiological progenitor cells [8].

IGF1 is a polypeptide that shares structural similarities with insulin and plays a significant role in regulating cell growth, proliferation, and differentiation [157–160]. In humans, a milder decrease in IGF-1 levels has been associated with rare genetic syndromes of hearing loss as well as ARHL [161]. IGF1R, a tyrosine kinase receptor that binds to IGF1, is expressed in cochlear sensory epithelia and SGNs [162, 163]. Analysis of IGF1R mutant mice has revealed abnormalities in cochlear structure, including a shortened cochlear duct, a reduced number of cochlear HCs, delayed maturation of the cochlear sensory epithelial, and defective formation of the semicircular canals [158]. IGF1 has been shown to have protective effects against excessive noise [164], ischemia [165], surgical invasion [166], and hearing loss induced by aminoglycosides [167]. In a clinical study of sudden sensorineural hearing loss (SSHL), IGF1 treatment was found to be more effective than glucocorticoid treatment [168, 169]. It has been reported that IGF1 has the potential to drive the regeneration of synapses between IHC and SGNs, which may explain its effect on hearing recovery [170]. Notably, Gao et al. also highlighted that exogenous IGF1 administration has a dual effect on ribbon synapses, promoting their recovery at low doses and impairing spontaneous recovery at high doses [171].

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF), is a highly specific pro-vascular endothelial growth factor that promotes increased vascular permeability, variability in the

extracellular matrix (ECM), migration and proliferation of endothelial cells, and vascularization. In addition, this growth factor directly affects neuronal cells, and dysregulation of its expression has been implicated in several neurodegenerative diseases [172]. Pericytes, which are multifunctional mural cells essential for maintaining the health of sensory hair cells and normal hearing, can promote SGN survival and neurite outgrowth by releasing exosomes containing VEGF-A [173]. Schwann cells that have been genetically modified to express S100A4 also significantly upregulate VEGF expression and provide neurotrophic support to SGNs [174]. Furthermore, Schwann and supporting cells produce a neurotrophic cytokine called macrophage migration inhibitory factor (MIF), which serves as a directional attractant and is required for ganglion genesis in the inner ear [175]. Notably, MIF-induced neurons derived from mouse ESC have the potential to replace lost or damaged SGNs [7].

Research into the protection and differentiation of SGNs using biomolecules has made some strides, but significant limitations and issues remain unresolved. Current studies predominantly employ in vitro experiments and animal models, which may not accurately reflect the situation in humans, necessitating more clinical research to validate laboratory findings. The mechanisms by which biomolecules like lactate influence SGN protection and differentiation are yet to be fully elucidated, requiring further investigation. Additionally, the lack of specific biomarkers for SGNs hinders research and clinical application. Challenges in drug development, such as designing small molecule drugs that can effectively deliver biomolecules to target cells and overcoming degradation and clearance issues within the body, must be addressed. The safety and potential side effects of using biomolecules for SGN protection and differentiation are also concerns, with long-term effects and side effects still poorly understood. In summary, despite progress in biomolecule research for SGN protection and differentiation, many deficiencies and challenges require resolution. Further research and methodological innovation are essential to address these issues and advance the field.

Scaffolds

Scaffold materials

The decellularized ECM is a three-dimensional macromolecular network that lacks cells and is composed of various glycoproteins, including collagens, proteoglycans/glycosaminoglycans, elastin, fibronectin, and laminin. It acts as a biological scaffold to regulate the diverse biological functions of resident cells [176]. Evans et al. have found that laminin and fibronectin can provide guidance cues for the neurites of SGNs. They suggested that higher concentrations of laminins in the osseous spinal lamina promote SGN outgrowth towards the organ

of Corti, while low concentrations within the organ of Corti may slow neurite outgrowth. Fibronectin under each row of hair cells provides a stop or avoidance signal [177]. Santi et al. described a protocol for cochlear decellularization using sodium dodecyl sulfate (SDS) and sodium deoxycholate (SDOC). Their study showed that SDS treatment removes more cellular components than SDOC [178]. Afterward, Mellott et al. utilized SDS-treated decellularized cochleae as mechanical and biochemical scaffolds for incorporating human Wharton's jelly cells (hWJCs) exogenously [179]. The study revealed the differentiation of hWJCs into inner ear hair cells within the organ of Corti. The migration of hWJCs to the osseous spiral lamina site, with a negative presence of MYO7A, was also indicated in their staining results. Therefore, the possibility of its differentiation into neuron like cells cannot be ruled out. Although studies have not yet characterized the differentiation of hWJCs into SGNs, previous research has shown the potential of amniotic fluid MSCs (which also belong to fetal appendages) to differentiate into SGNs [109, 180]. Therefore, it is speculated that decellularized cochleae could serve as biomaterials to induce SGN regeneration.

Hydrogels show great promise as scaffolds for guiding neuronal growth. Wille et al. Coated the fibers with heparan sulfate (HS), an extracellular matrix component, to mimic the natural extracellular matrix. By incorporating BDNF into these coated fibers, they achieved a sustained release of neurotrophins for an extended period of 110 days [131]. Cell-based drug delivery is another approach for the long-term treatment of inner ear neurons. CI can convert the encoded complex multi-frequency speech information into electrical signals that are then precisely transmitted to SGNs, enabling the treatment of patients with severe to profound sensorineural deafness. Considering the lifelong requirement of CI implantation, Scheper et al. used ultra-high viscous alginate (UHV-alginate) to encapsulate transgenic MSCs on CIs, enabling long-term administration of BDNF and inhibiting SGN degeneration [133]. The number and function of residual SGNs are the most critical limiting factors for effective CI implantation. Hydrogels have been employed to encapsulate genetically modified Schwann cells, which overexpress BDNF and NT-3, resulting in a significant improvement in SGN survival [181]. Purcell et al. implanted NSCs encapsulated in alginate hydrogel to promote the integration of prostheses with the surrounding brain tissue [182]. However, after 6 weeks of implantation, an obvious immunoinflammatory response and gliotic scar hyperplasia were observed, likely due to the degradation of the alginate hydrogel and death of seeded cells. Therefore, degradable nerve electrode interface materials need to have a controlled degradation rate to accommodate the re-formation of ECM. Based on this

consideration, researchers have utilized degradable poly (vinyl alcohol) (PVA) to form biocompatible hydrogels that can be tailored according to the desired degradation time frame and bioactivity [183, 184]. Additionally, the utilization of hydrogel coatings on the surface of CI electrodes has been shown to suppress the mechanical properties of hard electrode arrays and mitigate the generation of resistive scar tissue. Notably, hydrogels allow for optimal connectivity of water and ions in salt solutions, which is the basis for cell survival within them, but also pose the problem of hydrogel swelling. This swelling leads to increased distance between the electrode and target tissue, as well as relative displacement, and delamination from the electrode interface [185]. Faveri et al. reported that fibrin hydrogels exhibited less swelling than other hydrogels under same experimental conditions [186]. Meanwhile, Chang et al. proposed the integration of nanofibrillar cellulose (NFC) hydrogel as a mechanical scaffold with a Scala's squamous epithelium to create a supportive stem cell niche for exogenous human embryonic stem cell-derived otic neuronal progenitors (hESC derived ONPs) [187]. NFC hydrogels can mimic the ECM of native tissues in terms of fiber size and mechanical properties, enabling a feasible and sustainable microenvironment for exogenous cell growth [188, 189]. Chang et al. proposed a 3D polyhedrin delivery system (PODS[®]) for several weeks of BDNF release to promote cell survival and guide stem cell differentiation toward neuronal lineages [187]. In addition, conductive hydrogels (CHs), which are a hybrid material composed of a conductive polymer and a hydrogel, can be engineered to swell by adjusting the ratio of components. CHs often exhibit superior charge storage capacity, lower impedance, and no evidence of delamination or degradation [190, 191]. For example, Hassarati et al. coated olfactory ensheathing cells (OECs) in gelatin and sericin modified CHs, and the coated OECs supported the outward growth of nerves while crossing the glial scar tissue [192].

Physical stimulation

Electrical activity plays a crucial role in neurodevelopment [193]. Chronic electrical stimulation within the cochlea not only modulates neuronal excitability but also influences the maturation and synchronization of neuronal networks, thereby alleviating the onset of neuronal degeneration [194]. Graphene, a two-dimensional carbon atom monolayer, has been used in many biomedical fields due to its unique electrical, mechanical, and thermal characteristics and high biocompatibility [195]. It has the potential to manipulate stem cell fate, not only accelerating the differentiation of MSCs [196–199], but also effectively and self-organizing the differentiation of NSCs into neurons [200, 201]. Guo et al. suggested that graphene enhances the differentiation of NSCs into

neurons by altering passive and active bioelectric membrane properties through an internal electric field [202]. However, it appears that electrical stimulation alone does not facilitate the regeneration of SGNs. Considering this, they investigated the effects of cochlear implant-based electroacoustic stimulation on the proliferation and differentiation abilities of NSCs seeded on graphene [203]. The results showed that low-frequency electroacoustic stimulation (188–313 Hz) could significantly promote the proliferation and differentiation of NSCs on graphene into neurons. Notably, high-frequency stimulation (6,938–7,938 Hz) induced the death and apoptosis of NSCs. In addition, Liao et al. developed a cochlear implant electroacoustic stimulation (EAS) system combined with conductive $Ti_3C_2T_x$ MXene-matrigel to regulate the biological behaviors of SGNs [204]. $Ti_3C_2T_x$ MXene is a two-dimensional nanosandwich structure formed by transition metal element graphene oxide and carbon element, which can be variably functionalized through different surface modifications. The most recent findings demonstrate that primary mouse NSCs cultured on $Ti_3C_2T_x$ MXene display enhanced biological activity and neural network synchrony, characterized by longer neuronal processes, and increased branchial points and branch tips [205, 206]. Matrigel, also known as cultrex, or EHS martrix, is a highly bioactive tumor extract containing all components present in the basement membrane with the ability to promote cell survival and differentiation, resulting in enhanced graft survival *in vivo* and thus repair of damaged tissue [207]. Matrigel at concentrations higher than 4 mg/mL formed gels at 24–37°C [208]. Perny et al. used matrigel as a mechanical scaffold to achieve robust differentiation of mouse ESCs into ear sensory neurons by individually regulating signaling pathways such as transforming growth factor beta (TGF β), bone morphogenetic protein (BMP), and by progressively adding FGF2, BDNF, and NT-3 to mimic important steps of inner ear development [123]. The results showed better biocompatibility of $Ti_3C_2T_x$ MXene-matrigel from Liao et al. *In vivo*, SGNs coated in $Ti_3C_2T_x$ MXene-matrigel exhibited longer neuronal processes, larger growth cone areas, and filopodia in low-frequency EAS systems, and promoted the formation and maturation of neuronal networks [204].

Magnetic nanoparticles, another kind of common nanomaterial in biomedical fields, exhibit remarkable superparamagnetism in addition to large specific surface area, good surface modification [209, 210]. Superparamagnetic iron oxide nanoparticles (SPIO) exhibit good adhesion and excellent biocompatibility at concentrations below 500 μ g/mL. SPIO with static magnetic field (SMF) could promote NSCs proliferation, but high-intensity SMF (145 \pm 10 mT) inhibited the expansion ability of NSCs [211]. SPIO coated with poly-L-lysine can be

internalized by SGNs and shows no cytotoxicity at concentrations below 300 μ g/mL. SPIO internalized with or without external SMF could promote neurite extension by promoting the development of SGN growth cones. Moreover, SPIO could also physically direct SGN orientation and neurite outgrowth by regulating cell migration in the presence of external SMF [212]. Xia et al. performed surface modification based on Fe_3O_4 nanoparticles to form $Fe_3O_4@SiO_2$ nanoparticles and formed magnetic nanochains under static magnetic field induction [213]. Under the guidance of magnetic nanochains, the seeded NSCs exhibited good alignment, and neural stem cell-derived neurons showed good directional growth with the orientation of nanochains. In addition to SMF, the surface topography of the material is important for neuronal adhesion and guidance of neurite outgrowth [214, 215]. The surface topography of magnetic nanochains may also be involved in the active guidance of neurites. In addition, its coated silica nanoparticles can also serve as an important nonviral delivery system for retrograde transport in axons [216].

The studies of scaffolds for the protection and differentiation of SGNs has made strides, yet several challenges and limitations remain. Key issues include the inherent properties of biomaterial scaffolds, such as mechanical strength, degradation rate, and biocompatibility, which can affect SGN survival and function. Ensuring compatibility with neural tissue and promoting the desired cellular responses are critical challenges. Additionally, the precise control of axon guidance and the development of scaffolds that effectively support regeneration are technical hurdles. The complex interaction between SGNs and scaffolds influences cell behavior, highlighting the need for understanding and manipulating these interactions to enhance cell attachment, proliferation, and differentiation. Long-term performance degradation, host integration, and xenogeneic reactions are also important considerations, with potential negative impacts on SGNs. Lastly, scalability and cost-effectiveness are significant challenges, as successful translation from laboratory-scale production to clinical-scale manufacturing while maintaining cost-effectiveness is yet to be fully addressed.

Biohybrid neural interface

CI provides a standard treatment approach for patients with severe and profound sensorineural deafness by directly stimulating SGNs and transmitting auditory information from the periphery to brainstem nuclei. The quantity and quality of SGNs are the main factors limiting their effectiveness. The degenerative deformation of SGNs follows a sequence from distal to central and primarily affects the peripheral ends, leading to the formation of “electrode-neuron gaps” that reduce the spatial selectivity of the electrodes. A biohybrid implant involves

the integration of cells with an electrode array, which, when implanted, integrates into the host tissue to form a biohybrid neural interface facilitating communication between the electrode and tissue. These integrated cells function through two primary strategies: either by forming new tissues to replace the damaged host tissue or by providing a favorable microenvironment for the damaged host cells [91, 92]. One of the benefits of the biohybrid neural interface is the induction of neuronal growth, which reduces the distance between the electrode array and SGNs. This reduction in distance allows for a decrease in the stimulus intensity from the electrode array and reduces the activation of neighboring neurons, ultimately improving the spatial selectivity and performance of CIs in low signal-to-noise ratio environments [217]. Considering the timescale of CI implantation, foreign body reaction stands as a crucial challenge. The body's response to the implant involves an inflammatory reaction, where factors like the generated reactive oxygen species can directly harm the implant and surrounding tissues. Prolonged inflammation leads to the formation of a fibrotic tissue layer on the electrode surface, physically separating it from the surrounding tissue, and thereby gradually deteriorating the electrode-tissue interface [218]. Strategies involving silicone fibers to create intricate microscale surface topography for CI electrode contact areas or using coated CI electrodes such as protein-repellent polymers or conductive hydrogels, prove

beneficial in preventing fibroproliferation and reducing inflammatory reactions [219, 220]. However, over time, the encapsulated implant requires an increased amount of charge to stimulate the target tissue, eventually losing efficacy. As shown in Fig. 3B, Goding et al. proposed enhancing the integration of exogenous neural cells onto the electrode surface by enveloping them with an additional layer of degradable biosynthetic hydrogel on the surface of a non-degradable conductive hydrogel coating [221]. Their findings demonstrated that the additional non-conductive layer did not compromise the electrical properties of the electrode, and the bilayer structure notably improved interfacial flexibility. Glial cells encapsulated in a degradable hydrogel layer exhibited high bioactivity and produced ECM proteins exhibiting the potential to replace degradable hydrogels. However, the survival of neural precursor cells embedded in hydrogel remains insufficient, influenced by the harvesting technique. In addition, the biohybrid neural interface proves effective in addressing interface instability caused by the continued movement of body tissue under chronic implantation conditions [222].

The biohybrid neural interface can be classified into four primary types, as shown in Fig. 3A. The commonly used type of tissue engineering application in the inner ear is the cells-on-electrode approach. Wise et al. conducted an *in vivo* experiment where neonatal deaf cats were implanted with choroid plexus cells

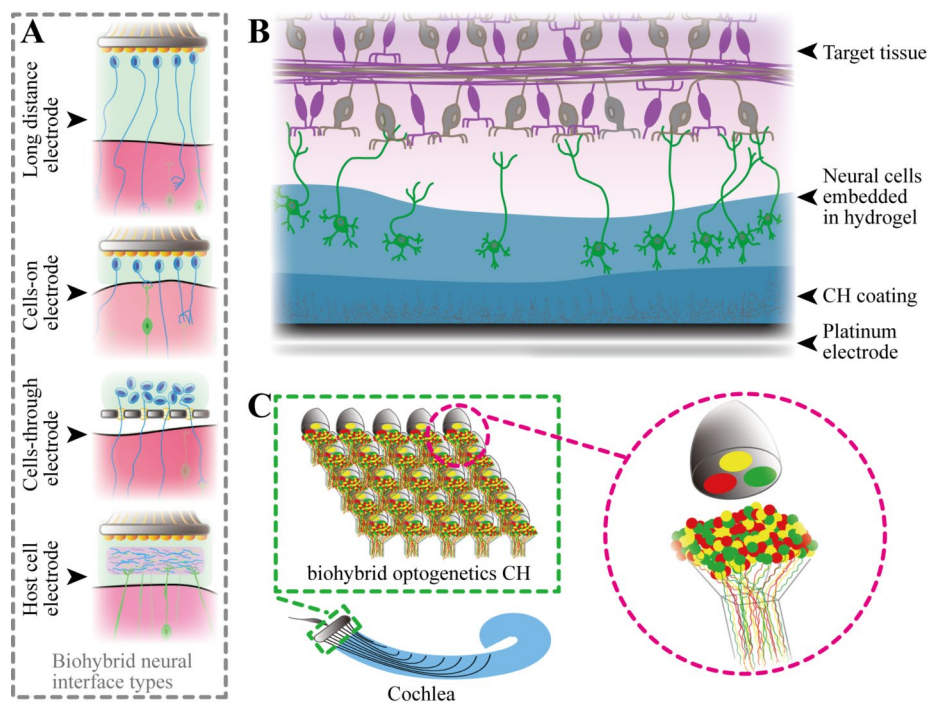


Fig. 3 (A) Four types of biohybrid neural interfaces. (B) Schematic representation of synaptic interfaces between an electrode and target tissue produced from integrating living neural cells into tissue-engineered hydrogel coatings. (C) Cochlear implant model based on biohybrid optogenetics with long-distance cell electrodes

coated on CI electrodes to achieve long-term neurotrophins (NTs) supply and prevent loss of SGNs [223]. The results showed that NTs treatment alone, unlike chronic electrical stimulation alone, increased the density of SGN peripheral protrusions and prolonged the survival cycle of SGNs in the upper basal cochlear region. Combining NTs and ES yielded superior therapeutic outcomes, as evident by the regrowth of peripheral processes in the scala media and scala tympani, which increased the potential for direct contact between peripheral processes and a CI electrode array. Building upon similar considerations, Schepers et al. encapsulated transgenic MSCs in ultra-high viscous alginate to achieve long-term administration of BDNF for maintaining the number and excitability of SGNs [133]. Their findings indicated that coating MSCs encapsulated in alginate onto CI significantly prevented the pathology and death of SGNs. Additionally, the protective efficacy of SGNs was investigated by directly injecting alginate MSCs into the scala tympani; however, this injection-based method did not showcase the effect of preventing SGN degeneration. Brant et al. proposed a cochlear implant model based on biohybrid optogenetics with a long-distance cell electrode (Fig. 3C) [217]. Their proposal involved controlling exogenous transgenic neuronal somata using an optrodecapable of emitting three wavelengths. The axons of these neurons could extend along the cochlear topology and establish synaptic connections with native SGNs or project along the eighth nerve to various regions of the auditory brainstem, enabling treatment for deafness. The utilization of host cell-based electrodes in the study of hearing loss is not as well-established, partially due to the involvement of decellularized tissues derived from the host. In the field of peripheral nerve regeneration, decellularized muscle tissue has been extensively studied and shown to promote functional axonal regeneration and restoration of locomotor activity [224, 225]. Studies on decellularized ear tissue have demonstrated its potential as a bioscaffold for stem cell differentiation [178, 179, 226], but the sourcing decellularized ear tissue from the host presents challenges. Therefore, exploring alternative ear tissues of host or non-host origin could be considered. This biohybrid interface also highlighted the attraction of native neurons to the cell-coated electrode surface to establish tight coupling. Drawing on the principle that Schwann cells and supporting cells can produce directionally inducible neural cytokines (such as MIF), Ramamurthy et al. coated CI electrodes with MIF-expressing Schwann cells, successfully inducing directional growth of SGNs and establishing tight coupling with CI [7].

Conclusions and perspectives

The field of tissue engineering has emerged as a promising avenue for the protection and regeneration of SGNs, which are crucial for hearing. This review article encapsulates the latest advancements and initiatives within the realms of biotechnology and tissue engineering that are aimed at safeguarding and revitalizing SGNs. It delineates the injury mechanisms that impact SGNs, the identification and application of seed cells for the replacement and protection of these neurons, the role of biomolecules in orchestrating seed cell differentiation and the rescue of remaining SGNs, and the utilization of scaffold materials and physical stimulation techniques for the reconstruction of inner ear tissue and the restoration of hearing function. Furthermore, the article also highlights the progress in the development of biohybrid neural interfaces, which are anticipated to play a significant role in future hearing reconstruction therapies.

One of the key advantages of tissue engineering strategies for SGN protection and regeneration is their potential to overcome the limitations of current treatments for SNHL, which primarily focus on hearing aid amplification and cochlear implants. Tissue engineering offers a paradigm shift by addressing the root cause of SNHL - the damaged or degenerated SGNs. By regenerating these neurons and restoring the auditory neural pathways, tissue engineering holds the promise of providing a more natural and effective treatment for SNHL.

Despite the promise, there are several challenges that need to be addressed to translate tissue engineering strategies into clinical applications. Firstly, strategies to enhance the survival rate of implanted stem cells are essential to ensure robust regeneration. Secondly, the biocompatibility of the scaffolds used for supporting the regenerated neurons needs to be improved to promote their survival and functionality. Lastly, the identification of the optimal temporal window for the activation of molecular cues is crucial to orchestrate the regeneration process effectively.

To overcome these challenges, a multidisciplinary approach that integrates basic scientific research, engineering innovation, and clinical expertise is indispensable. A profound understanding of the intricate anatomical framework of the cochlea, including the spatial organization of its cellular constituents, is required to design and implement successful tissue engineering strategies. Additionally, unraveling the molecular underpinnings that govern cochlear development and the temporal regulation of gene expression networks will provide valuable insights for the development of effective treatment approaches.

In conclusion, tissue engineering strategies for SGN protection and regeneration offer a novel and promising avenue for the treatment of SNHL. While there are

significant challenges that need to be addressed, the potential to regenerate damaged auditory neural pathways and restore hearing function makes this field worth pursuing. Future research should focus on developing innovative strategies to improve the survival and functionality of implanted stem cells, enhancing the biocompatibility of scaffolds, and identifying the optimal temporal window for molecular cue activation. With advancements in basic scientific research, engineering innovation, and clinical expertise, tissue engineering holds the potential to revolutionize the field of hearing restoration and provide new hope for individuals suffering from SNHL.

Abbreviations

SGNs	Spiral ganglion neurons
SNHL	Sensorineural hearing loss
HCS	Hair cells
IHCs	Inner hair cells
CI	Cochlear implant
TTS	Temporary threshold shift
PTS	Permanent threshold shift
SPL	Sound pressure level
NIHL	Noise induced hearing loss
ARHL	Age-related hearing loss
ROS	Reactive oxygen species
NF-Kb	Nuclear factor kappaB
AABs	Aminoglycoside antibiotics
CMV	Cytomegalovirus
ABR	Auditory brainstem response
LCMV	Lymphocytic choriomeningitis virus
HIV	Human immunodeficiency virus
HSV	Herpes simplex virus
POU3F4	POU class 3 homeobox 4
SLC17A8	Soluble carrier family 17, member 8
VGLUT3	Vesicular glutamate transporter-3
SC	Supporting cell
GJB2	Gap junction protein, beta 2
OTOF	Otoferlin
NSCs	Neural stem cells
ESCs	Embryonic stem cells
iPSCs	Induced pluripotent stem cells
MSCs	Mesenchymal stem cells
NNE	Nonneuronal ectoderm
PPE	Preplacodal ectoderm
ONP	Otic neuron progenitor
OEP	Otic epithelial progenitors
L-Gln	L-Glutamine
DFNB	Dulbecco's Modified Eagle's Medium (DMEM) with Ham's F12 and N2/B27
HFF	Human foreskin fibroblasts
NBM	Neurobasal A with 1% N2, 2% B27, 2 mM l-glutamine and 0.5% penicillin/streptomycin
CDM	Chemically defined medium
kDMEM	Knockout Dulbecco's modified Eagle's medium
KOSR	Knockout serum replacement
NEAA	Nonessential amino acids
BME	β -mercaptoethanol
pps	Pulses per second
CN	Cochlear nucleus
NST	Nucleus of the solitary tract
SYP	Synaptophysin
PRPH	Peripherin
NTFs	Neurotrophic factors
NGF	Nerve growth factor
NT-3	Neurotrophin 3
Oct3/4	POU class 5 homeobox 1

Sox2	SRY-box 2
c-Myc	Myelocytomatosis oncogene
Klf4	Kruppel family member 4
Neurog1	Neurogenin 1
Eya1	Eyes absent 1
Six1	SIX homeobox 1
bFGFs	Basic fibroblast growth factors
GATA3	GATA binding protein 3
VGLUT1	Vesicular glutamate transporter 1
BMSC	Bone marrow stromal cells
Ni-hMSCs	Neural-induced human MSCs
WJ MSCs	Wharton's jelly MSCs
GDNF	Glial cell line-derived neurotrophic factor
DPOAE	Distortion product otoacoustic emission
Atoh1	Atonal Homolog 1
HL-MSCs	Human limbal MSCs
DPSCs	Dental pulp stem cells
TrkB	Tyrosine kinase receptor B
NT-4/5	Neurotrophin-4/5
p75 ^{NTR}	p75 neurotrophin receptor
Trk	Tropomyosin receptor kinase
TrkC	Tyrosine kinase receptor C
PLC-gamma	Phospholipase-c-gamma
oe NSCs	Olfactory epithelial neural stem cells
pnt-1	Pan neurotrophin 1
PPAR γ	Peroxisome proliferator-activated receptors
FGF2	Fibroblast growth factor 2
RA	Retinoic acid
EGF	Epidermal growth factor
SSH1	Sudden sensorineural hearing loss
VEGF	Vascular endothelial growth factor
VPF	Vascular permeability factor
ECM	Extracellular matrix
MIF	Migration inhibitory factor
SDS	Sodium dodecyl sulfate
SDOC	Sodium deoxycholate
hWJCS	Human Wharton's jelly cells
HS	Heparan sulfate
UHV	Ultra-high viscous
NFC	Nanofibrillar cellulose
CHs	Conductive hydrogels
OECs	Olfactory ensheathing cells
EAS	Electroacoustic stimulation
TGF β	Transforming growth factor beta
BMP	Bone morphogenetic protein
SPIO	Superparamagnetic iron oxide nanoparticles
SMF	Static magnetic field
LE	Living electrode
NTs	Neurotrophins

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Author contributions

RJC, JQS, TTL, LT and YNH defined the focus of the review. BZ and YNH summarized studies. BZ drafted the manuscript. HLD, SYH, LR and HC participated in some parts of the final manuscript. BZ, YNH, YSW, XG, SSZ and QYC revised the manuscript. All authors reviewed the final version of the manuscript. All authors read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

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Not applicable.

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Competing interests

The authors declare no competing interests.

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